

## Product Description

The capping efficiency is a critical quality attribute (CQA) for mRNA vaccine therapeutics and constitutes essential validation data required for the regulatory submission of mRNA-based products. Currently, the determination of capping efficiency is primarily conducted via liquid chromatography-mass spectrometry (LC-MS). There is a difference on the order of hundreds in molecular weight between mRNA with a cap structure and uncapped mRNA. Compared to the molecular weight of intact mRNA, which is on the order of hundreds of thousands, it is challenging to precisely analyze the difference between capped and uncapped samples. Therefore, analyzing capping efficiency first requires processing the long mRNA fragments into smaller segments, thereby transforming the task of finding a difference on the order of hundreds within hundreds of thousands into finding a difference on the order of hundreds within thousands. The mRNA Capping Efficiency Analysis Pre-treatment Kit developed by Baorui Biotech can accurately and efficiently process mRNA samples. The processed samples can be directly used for LC-MS analysis, facilitating rapid assessment of sample capping efficiency. This kit is suitable for the pre-treatment of mRNA samples with different capping methods and various cap structures.

## Components

	Components	Volume	Storage
Box 1	M Reagent	40 $\mu$ L	-20 $\pm$ 5 $^{\circ}$ C
	10 $\times$ M Buffer	200 $\mu$ L	
	Buffer 1	1 mL	
Box 2	Magnetic Beads	1 mL	2-8 $^{\circ}$ C
	Loading Buffer	200 $\mu$ L	
	Buffer 2	18 mL	
	Buffer 3	1 mL $\times$ 2	
	EB Buffer	1 mL	

## Storage

Box 1 is stored at -20 $\pm$ 5 $^{\circ}$ C and Box 2 at 2-8 $^{\circ}$ C. Do not freeze-thaw the magnetic beads.

## Materials

Required: Consumables: 200  $\mu$ L PCR tubes, 1.5 mL nuclease-free microcentrifuge tubes.

Reagents: Nuclease-free water, Probe.

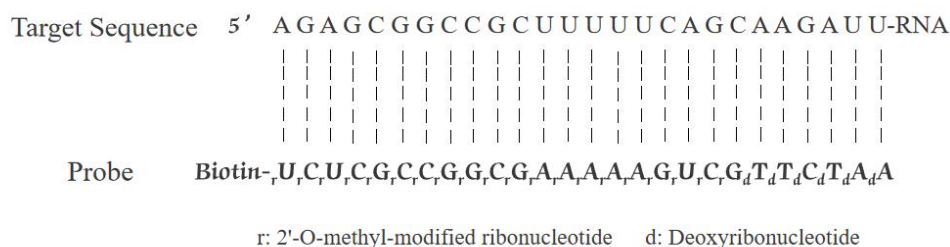
Optional:

Reagents: Methylene blue, Sodium acetate, 10 $\times$  TBE buffer, 45% Acr (43.4 g acrylamide plus 1.6 g bis-acrylamide, brought to 100 mL with ultrapure water, filter and store protected from light at 4 $^{\circ}$ C), Urea, Tetramethylethylenediamine (TEMED), 10% Ammonium persulfate (AP).

## Protocol

### 1. Probe Design and Synthesis

This kit does not provide specific probes. Probes need to be designed based on the target sequence. The designed probe must consist of three parts (as shown in the figure): a 3' end sequence of 20-25 ribonucleotides complementary to the 5' end of the target sequence. To prevent probe degradation, modified ribonucleotides can be used in this region, with 2'-O-methyl modified nucleotides recommended; followed by a sequence of 6 deoxyribonucleotides complementary to the target sequence, which requires no modification; and finally, a 3' end modified with Biotin. A single synthesis of 1 OD of probe is sufficient for the number of reactions specified by this kit. For recommended suppliers for probe synthesis, please contact technical support.



## 2. Digestion

2.1 For the synthesized probe in dry powder form, briefly centrifuge the tube before opening to collect the contents at the bottom. In a clean bench, dissolve the probe in an appropriate volume of Buffer1 to a final concentration of 100 pmol/ $\mu$ L. Note: Refer to the probe synthesis label for the specific volume of Buffer1 required. The dissolved probe can be aliquoted and stored at -80°C.

2.2 Using a 200  $\mu$ L nuclease-free PCR tube, add 150 pmol of RNA, followed by 150 pmol of the probe. Mix gently by pipetting.

Note: Rough calculation for 50 pmol RNA amount: RNA quantity (ng) = 0.05 nmol  $\times$  330 g/mol  $\times$  X nt, where X = number of RNA bases. Ensure the total volume in each tube is less than 60  $\mu$ L in this step. For large-volume reactions, an 8-tube strip can be used, and the contents can be combined after incubation.

2.3 Set up the PCR program: 95°C, 5 min; 65°C, 2 min; 55°C, 2 min; 40°C, 2 min; 22°C, 2 min.

2.4 After the reaction is complete, add 3  $\mu$ L of M Reagent and the corresponding 10x M Buffer to the mixture. Incubate at 37°C for 2 hours.

## 3. Urea-PAGE Digestion Verification (Optional)

### 3.1 Reagent Preparation

3.1.1 Staining Solution Preparation: 2 g/L Methylene Blue + 0.4 M Sodium Acetate (Sodium Acetate pH=4.7).

Note: The staining solution can be reused multiple times (>10 times). Handle with care due to the slight toxicity of methylene blue during operation.

3.1.2 Prepare a 21% Urea-PAGE gel according to the following formulation.

Components	Volume
45%Acr	10 mL
Urea	9.60 g
10 $\times$ TBE	2 mL
10%AP	140 $\mu$ L
TEMED	14 $\mu$ L

### 3.2 Sample Processing

Control group: 4.5  $\mu$ L nuclease-free water + 0.5  $\mu$ L probe + 5  $\mu$ L Loading Buffer.

Experimental group: 5  $\mu$ L enzyme-digested sample + 5  $\mu$ L Loading Buffer.

Mix the sample thoroughly and denature at 70°C for 5 minutes, then immediately place on ice.

### 3.3 Electrophoresis

Pre-run at 260 V for 26 minutes. After loading the samples, run at 260 V for 2 hours. Stain for 10 minutes, then destain with ultrapure water overnight.

Note: Samples can be temporarily stored at -20°C at this stage.

## 4. Recovery

### 4.1 Magnetic Bead Preparation

After thoroughly mixing the magnetic beads, transfer 70  $\mu$ L of beads into a nuclease-free microcentrifuge tube. Place the tube on a magnetic stand for magnetic separation and remove the supernatant. Then, remove the tube from the magnetic stand.

### 4.2 Magnetic Bead Washing

Add 200  $\mu$ L of Buffer 2 to the tube. Mix thoroughly, then place the tube on the magnetic stand for magnetic separation and remove the supernatant. Repeat this washing step twice.

#### 4.3 Sample Binding

Add the entire digested sample to the tube. Mix thoroughly. If necessary, use a mixer to mix at room temperature for 30 minutes. After mixing, place the tube on the magnetic stand for magnetic separation and remove the supernatant. Then, remove the tube from the magnetic stand.

#### 4.4 Washing

Add 200  $\mu$ L of Buffer 2 to the tube. Place the tube on the magnetic stand for magnetic separation and remove the supernatant. Repeat this washing step four times. Then, remove the tube from the magnetic stand.

#### 4.5 Secondary Washing

Add 100  $\mu$ L of pre-chilled Buffer 3 and mix. Place the tube on the magnetic stand for magnetic separation and remove the supernatant. Then, remove the tube from the magnetic stand.

Note: The supernatant from this step can be temporarily stored at  $-80^{\circ}\text{C}$  to prevent sample loss.

#### 4.6 Elution

Add 25  $\mu$ L of pre-warmed ( $80^{\circ}\text{C}$ ) EB Buffer to the beads and mix thoroughly. Heat at  $80^{\circ}\text{C}$  for 4 minutes. Place the tube on the magnetic stand for magnetic separation and transfer the supernatant to a labeled nuclease-free microcentrifuge tube. Repeat this elution step twice. Finally, combine the supernatants from both elutions. Place the combined eluate on the magnetic stand again to adsorb and ensure removal of any residual magnetic beads.

Note: The presence of magnetic beads in the prepared sample can damage detection instruments and chromatography columns. To avoid this, avoid touching the beads when aspirating the supernatant. A sample volume of 25  $\mu$ L or more is sufficient for subsequent applications.

#### 5. Urea-PAGE Digestion Verification

To verify the processing efficiency of this kit for capped samples and ensure the reliability of subsequent sequencing results, use Urea-PAGE to verify the recovered fragments. For the Urea-PAGE operation procedure, please refer to the "3 Urea-PAGE Digestion Verification" section in this chapter.

### Notes

1. Store the components of the kit as required: M Reagent, 10 $\times$ M Buffer, and Buffer 1 should be stored at  $-20^{\circ}\text{C}$ ; all other reagents should be stored at  $4^{\circ}\text{C}$ . Note that the magnetic beads should not be subjected to freeze-thaw cycles.
2. The magnetic beads, reagents, and other materials provided in this kit have undergone extensive screening and validation. To ensure processing efficacy, do not arbitrarily substitute the materials supplied with the kit, as this may compromise experimental results.
3. Step 3 is optional. It can be used to assess the effectiveness of enzymatic digestion, helping to decide whether to further optimize digestion conditions or proceed with product recovery. To ensure the quality of subsequent sequencing or analysis, processed samples should be verified by Urea-PAGE.
4. Samples processed with this kit should be analyzed promptly. Storage time should ideally be less than one week. Uncapped samples are prone to degradation during prolonged storage, which may lead to an overestimation of capping efficiency in subsequent tests.
5. Observe appropriate personal protective measures during the experiment.
6. Please mix thoroughly before use. Avoid repeated freezing and thawing.